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THE PATHOGENICITY OF *STREPTOCOCCUS LACTICUS*.*

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It has been shown by many observers that streptococci frequently occur in milk and Escherich[1] is of the opinion that infants fed on cow's milk have especially large numbers of streptococci in the intestines. These streptococci he found fatal to mice in some instances. Milk streptococci have been found pathogenic by some but not all observers. Beck[2] injected the centrifugal residue of milk into guinea-pigs which then died of peritonitis caused by streptococci. Pure cultures of this streptococcus in mice brought about a fatal general infection. Rubbing on the ear produced reddening of the skin in some instances. Bergey[3] injected streptococci from milk into the skin of the ears of rabbits with the result that slight local lesions developed. The amount injected is not stated. Lammeris and Harrevelt[4] recovered a streptococcus from milk which had been the cause of serious stomach troubles and diarrhea in adults, but found it non-pathogenic for rabbits and guinea-pigs. Park and Holt[5] stated that 40 per cent of all bacteria injected into guinea-pigs proved pathogenic, but whether streptococci were among these is not stated. V. Freudenreich[6] inoculated rabbits with streptococci from milk with negative results. Hölling[7] inoculated 11 mice with as many strains of *Strept. lacticus*. Three of these died. Brüning[8] found streptococci in 93 per cent of Leipzig milk which produced fatal general infections in mice but did not affect guinea-pigs. Savage[9] obtained negative results from milk streptococcus

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* Received for publication October 20, 1906.

[1] *Jarhb. f. Kinderheilk.*, 1899, 49, p. 137.

[2] *Deutsche Vierteljahrsschr. f. öffentl. Gesundheitspfl.*, 1901, 32, p. 430

[3] *Am. Med.*, 1901, 1, p. 122..

[4] *Zeitschr. f. Fleisch u. Milch Hyg.*, 1901, 11, p. 114.

[5] "Upon the Results with Different Kinds of Pure and Impure Milk in Infant-feeding in Tenement Houses, etc.," *Archiv. of Pediatrics*, 1903.

[6] *Centralbl. f. Bakt.*, Abth. 2, 1904, 13, p. 281.

[7] *Inaug. Dissert.*, Bonn, 1904.

[8] *Jahrb. f. Kinderheilk.*, 1905, 12, p. 1.

[9] *Jour. Hyg.*, 1906, 6, p. 123.

injections. Müller[1] stated that the speed of coagulation of milk streptococci and pathogenic streptococci differs materially, but that there is no difference in agglutination or hemolysis. Kaiser[2] found streptococci in 76.6 per cent milk after ruling out diplococci and short chains.

It is probably true, then, that streptococci freshly isolated from milk, apart from those originating from mastitis, do not possess high virulence. I have shown[3] that *Strept. lacticus* occurs constantly in milk and since the morphological and cultural characters of this variety of streptococcus on ordinary media are identical with those of *Strept. pyogenes,* it seemed likely that the virulence might be increased by successive passages through animals.

Experiments along this line have been carried on by the writer with rabbits, the rabbit being chosen because of its susceptibility to streptococcus infection.[4] The following plan was pursued. Various cultures of *Strept. lacticus* were "rejuvenated" by passing through three tubes of serum broth, and 2 c.c. of the last culture injected, either subcutaneously or intravenously, into a rabbit. From the lesions produced the organism was recovered after two days, and 2 c.c. of a 24-hour-old broth culture then injected into another animal. From each of the animals cultures were prepared and inoculated into ordinary broth and 2 per cent lactose broth, litmus milk, and slant agar, in order to find whether any cultural difference from the original appeared. Microscopical examinations were also made in each instance. The following strains were employed:

1. *Strept. lacticus* isolated from certified milk by the writer.
2. Another strain of the same organism. These two cultures were selected at random from a large number, and were typical of *Strept. lacticus* (*B. lactis acidi,* Leichmann).
3. *Strept. lacticus* obtained from Professor Harding, N. Y. Agricultural Experiment Station, Geneva, N. Y. This organism was to all appearances identical with the two previous ones in cultural and morphological characters.

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[1] *Archiv f. Hyg.*, 1906, 56, p. 90.

[2] *Ibid.*, p. 51.

[3] *Jour. Infect. Dis.*, 1906, 3, p. 173.

[4] V. Lingelsheim in Kolle and Wassermann, *Handbuch der Pathogenen Mikroorganismen*, 1903, 3, p. 350

4. *Strept. lacticus* from a commercial starter.

5. *Strept. lacticus* also from a commercial starter. These two cultures, 4 and 5, agreed with typical *Strept. lacticus* in morphological and cultural characters.*

As controls and for comparison three strains of *Strept. pyogenes* were injected into rabbits in the same manner and in the same doses. Following are the sources of these cultures.

6. *Strept. pyogenes* isolated from the tonsil in a case of tonsilitis.

7. *Strept. pyogenes* isolated from a fatal case of peritonitis.

8. *Strept. pyogenes* isolated from a case of meningitis.†

These three strains possessed the usual cultural and morphological characters, but No. 8 coagulated milk very slowly.

After passage through animals the streptococci showed no noticeable cultural divergence from the original cultures. The only morphological difference was shown by *Strept. lacticus* No. 1, the original shape of this organism changing somewhat, and the individual cells in cultures from animals appearing rounder and smaller. Microscopical preparations from the lesions showed mostly diplococci and sometimes short chains. *Strept. lacticus* and *Strept. pyogenes* appeared alike.

A detailed description of the experiments follows:

1. *Strept. lacticus* (from milk): This strain was passed through 10 rabbits successively. A uniform amount each time (2 c.c.) was injected subcutaneously with the usual antiseptic precautions. The first rabbit developed an induration at the point of inoculation. The organism was recovered from the serous exudate after two days. The second rabbit was inoculated with a 24-hour-old broth culture of the recovered streptococcus in the same manner. It developed a small abscess. The pus was of a tough viscous consistency. The organism was recovered from this pus and a third animal inoculated with a culture therefrom. These methods of inoculation and recovery of the bacteria were pursued throughout all succeeding experiments. The pus pocket increased in size and rapidity of development with each succeeding rabbit. The fifth animal died in 29 days. Numerous pus centers were found under the skin, and purulent exudates were seen in the pleural and pericardial cavities. The ninth rabbit died in 16 days from a general streptococcus infection. There were fibrino-pur-

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* Seven commercial starters were obtained from the manufacturers and examined bacteriologically. Five of these consisted of practically pure cultures of *Strept. lacticus*, while two were not pure cultures but contained streptococci in large numbers. After preparing pure cultures from all starters by plating, they were studied and found to possess all the characters of *Strept. lacticus*. The streptococci obtained from two of the starters were very feeble, growing but poorly and coagulating milk much more slowly than *Strept. lacticus* usually does.

† These strains were kindly given me by Dr. H. T. Ricketts and Mr. J. E. Tyree, Department of Pathology, University of Chicago. They were isolated less than two weeks before the commencement of my experiments

ulent exudates in the pleural, pericardial, and peritoneal cavities, and numerous purulent foci under the skin. Streptococci were recovered from the pleural and peritoneal cavities, heart's blood, kidney, spleen, and subcutaneous pus. The 10th rabbit died after nine days. The lesions were similar to those described in the foregoing. All these rabbits lost progressively in weight and presented an emaciated appearance. Five out of the 10 rabbits inoculated gradually recovered. Several, at the time of writing, still have a subcutaneous pus pocket and their emaciated appearance gives rise to the conjecture that they may finally die. In two instances no lesions developed at the point of inoculation. These rabbits died after a lapse of four weeks with the phenomena of general infections of the same nature as described, except that no noticeable change took place at the point of inoculation.

The same streptococcus (No. 1) from the original culture was injected intravenously into a rabbit. The animal lost weight slowly and finally died after 25 days A large subcutaneous pus pocket had formed in the neighborhood of the hip on the right side, coincident with numerous pus foci of the same nature in other parts of the body. The pleural and peritoneal cavities were filled with a sero-fibrinous exudate and the pericardium showed a fibrino-purulent exudate. The organism was recovered from the pus, pleural, peritoneal, and pericardial cavities, and the heart's blood. From a broth culture prepared from the heart's blood a normal rabbit was injected intravenously and likewise a rabbit of the same weight, which had been previously inoculated subcutaneously with the same organism, but had recovered from the effects to all appearances. The former died in 17 days, the latter in six. The lesions were the same in both cases and identical with those described.

2. *Strept. lacticus* (No. 2, from milk): This strain produced no local effect. The animal after several days commenced to carry the head bent toward the left side, and when touched would walk around in a circle. When put on its back it regained its foothold only after several strenuous efforts. The animal seemed gradually to recover its normal condition, although it was considerably emaciated. Quite unexpectedly, after about six weeks, it died. Postmortem examination showed an enormously large amount of whitish semi-fluid pus in the pleural cavity. No other lesions could be detected. Streptococci were recovered from the pus and the heart's blood. Possibly the twisting of the head was due to an affection of the central nervous system by a toxin produced by the streptococcus. Similar observations have been recorded, among others by v. Lingelsheim.[1]

3. *Strept. lacticus* (from N. Y. Agricultural Experiment Station): The results of subcutaneous inoculation were quite similar to those described, excepting that the virulence increased somewhat more rapidly. The seventh rabbit died in 16 days, the eighth after 10 days.

4. *Strept. lacticus* (from a commercial starter): This organism seemed more virulent than any of the former three. The first rabbit developed a pus pocket at the point of inoculation of about an inch in diameter. The pus was of a thick, ropy consistency. An erysipelas-like reddening of the skin developed in the third rabbit at the point of inoculation. This reddening spread rapidly for four or five days, then came to a standstill, and finally disappeared. A large area of necrotic tissue formed in the fourth animal. This one also finally recovered. The seventh rabbit died on the ninth day after inoculation with the same postmortem picture as seen

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[1] *Loc. cit.*

in the other animals. Here also streptococci were recovered from the pleural and pericardial cavities and the heart's blood.

5. *Strept. lacticus* (from a commercial starter): This strain was injected subcutaneously but produced no local effect. The rabbit suffered from a nervous trouble similar to that described under No. 2. The animal carried the head bent over to one side but gradually seemed to recover. It died after several weeks. No postmortem lesions whatever could be detected.

6, 7, 8. *Strept. pyogenes:* The three varieties of *Strept. pyogenes* produced the same effect on rabbits as *Strept. lacticus*, except that death occurred in a shorter time. *Strept. pyogenes* from tonsilitis (No. 6) caused the sixth rabbit to die in seven days. *Strept. pyogenes* from peritonitis caused the third rabbit to die in eight days, and *Strept. pyogenes* from meningitis produced the death of the fourth rabbit in seven days. The lesions were in every respect identical with those brought about by *Strept. lacticus*. By a fortunate coincidence the 10th rabbit inoculated with *Strept. lacticus* (No. 1) and the third rabbit inoculated with *Strept. pyogenes* from peritonitis (No. 7) died at the same time, the former after nine, the latter after eight days. Postmortem examinations were made simultaneously. The pathological conditions in the two animals were strikingly alike. Pus pockets under the skin in various places, the usual pericarditis, and the purulent exudate in the pleural cavity revealed identical conditions in both cases. Streptococci were recovered in both cases from the affected parts.

At the end of these experiments the different cultures from each of the animals were cultivated on slant lactose agar, plain and lactose broth, and litmus milk. It was noted that no change in the reactions on these media was effected by the passage through animals.

The conclusion seems justified that *Strept. lacticus*, by prolonged habitation in the body of the rabbit, is able to become virulent in large measure and approximate, if not equal, the virulence for this animal of typical *Strept. pyogenes* from human affections. It must be taken into consideration that the varieties of *Strept. pyogenes* employed in these experiments were recently isolated from pathological lesions. The streptococci from milk (Nos. 1, 2, and 3) had possibly lost in virulence by nine months' cultivation on artificial media. The organisms from commercial starters, although freshly isolated from the starters, probably had been in artificial cultivation for an indefinite length of time.

The negative results of inoculation experiments with milk streptococci by some observers may be explained in two ways. First: The lesions are often, especially in the beginning, insignificant and are easily overlooked. Second: Since lesions are insignificant with large doses it is quite possible that small doses are unable to create any noticeable disturbances. Summarizing the results of the experiments described the following points seem established.

1. *Strept. lacticus* is closely related to *Strept. pyogenes,* not only morphologically and culturally, but also in pathogenic property.

2. By repeated passages of *Strept. lacticus* through rabbits its virulence is gradually increased. After five or more passages *Strept. lacticus* in subcutaneous injections becomes fatal in doses of 2 c.c. of 24-hour-old broth cultures. The same amount injected intravenously proved fatal to the first animal.

3. The lesions produced by *Strept. lacticus* in rabbits are of the same kind and extent as those produced by *Strept. pyogenes* from pathological conditions in human beings.

The writer acknowledges his obligation to Professor Edwin O. Jordan for advice and assistance in this work.